

OXYGEN UPTAKE AND RESPONSES TO RESPIRATORY STRESS IN SEA URCHINS¹

KJELL JOHANSEN² AND ROBERT L. VADAS³

*Friday Harbor Laboratories and Department of Zoology,
University of Washington, Seattle, Washington 98105*

In discussions of oxygen consumption in invertebrates it is common practice to classify animals as oxygen conformers or regulators. The conformers are often referred to as being respiratory-dependent whereas the regulators are independent. Such classifications imply that the animals either vary their oxygen uptake relative to the oxygen availability in the external medium, or they show some degree of regulation by maintaining oxygen uptake in spite of a decrease in the surrounding oxygen availability. An animal showing respiratory independence usually becomes respiratory dependent as the external oxygen tension (PiO_2) decreases. The PiO_2 at which this occurs is referred to as the critical oxygen tension. The concept of respiratory dependence implies that the animal is at the mercy of the existing environmental conditions. Although such classifications may serve to clarify similarities between various animals, they may obscure basic characteristics of animal energy economy and of important factors affecting interaction of the organism and its environment. We contend in opposition to current teaching that an animal's oxygen uptake must have a closer correlation with internal oxygen tensions than with the external oxygen availability. Recent advances in gas analyzing techniques have simplified the measurement of internal oxygen tensions. The present report is based on measurements of oxygen uptake (VO_2) of three species of sea urchins. Internal as well as external oxygen tensions were monitored as the animals were subjected to a variety of external conditions.

MATERIALS

Two of the three species of the genus *Strongylocentrotus* used in these experiments are extremely abundant members of the marine fauna in the vicinity of the marine laboratory at Friday Harbor, Washington. *S. droebachiensis* and *S. franciscanus* exhibit a marked subtidal zonation, with the former usually occupying the lower intertidal and the upper subtidal zones, while the latter is generally found several meters below zero tide. However, both species have been collected from depths as great as 35 meters and as intertidal individuals in slightly exposed areas. *S. purpuratus*, on the other hand, is mainly found intertidally in tide pools on very exposed, wave-beaten rocks. Natural and transplanted populations of *S. purpuratus*

¹ This work was supported by grant GB-4038 from the National Science Foundation, and by NSF grant G-20901 to the Friday Harbor Laboratories for support of the junior author.

² This work was done during the tenure of an Established Investigatorship of the American Heart Association.

³ Botany Department, University of Washington, Seattle, Washington 98105.

were located in limited areas around Friday Harbor. In addition, several collections were made from the exposed coast, at Mukkaw Bay.

All animals were stored in running, oxygenated, sea water for at least one week prior to use. Water temperatures varied from 9.5° to 10.5° C., and the animals were maintained under relatively low light conditions (about 40 foot candles). Upon completion of experiments, animals were returned to similar tanks and observed over a period of several weeks for any signs of ill effects as a result of the experimental procedures.

METHODS

Partial pressures of oxygen in the external water (PiO_2) and in the coelomic fluid ($PcfO_2$) were measured using a Beckman Spinco gas analyzer (model 160). A special micro-cuvette permitted duplicate analyses of all samples (sample size: 0.05 ml.). The oxygen electrodes were calibrated with known gas mixtures or solutions equilibrated to known gas composition. Repeated sampling of coelomic fluid was accomplished by means of polyethylene catheters (P.E. 90) chronically implanted through a small incision in the peristomial membrane with the catheter tip protruding into the large coelomic cavity. Leakage around the catheter was prevented by a purse string suture in the peristomial membrane. Measurements of PO_2 were made at a temperature corresponding to the prevailing temperature in the animal's immediate surroundings.

Oxygen uptake (VO_2) was measured using a closed system at constant temperature (10.0° C.) (Lenfant, 1961). The partial pressure of oxygen in the respirometer was slowly reduced by the oxygen consumption of the animals. The size of the respiration chamber was adjusted until a reduction in PiO_2 from 150 mm. Hg to 10–20 mm. Hg occurred within a 6–8 hour period. VO_2 was calculated as microliters O_2/g . wet weight/hour. Both PiO_2 and $PcfO_2$ were measured in successive samples during the oxygen uptake experiments. An integral part of the investigation involved sampling of coelomic fluid from animals in their natural habitats. Such samples were obtained while SCUBA diving and collected into greased glass syringes by a needle inserted into the coelomic cavity through the peristomial membrane. The samples were quickly brought to the laboratory for analyses of $PcfO_2$.

RESULTS

Figure 1 shows a plot based on the average of three experiments utilizing six animals. Oxygen uptake (VO_2) (left ordinate, open circles) and PO_2 in coelomic fluid ($PcfO_2$) (right ordinate, filled circles) are plotted against oxygen tension in the ambient water PiO_2 in a closed system. In addition, values of $PcfO_2$ obtained from animals in their normal habitats have been plotted as a function of the PiO_2 at which the samples were taken (open squares, Fig. 1).

In well aerated water there was always a gradient in PO_2 between the coelomic fluid and the ambient water (Figs. 1 and 2). Upon lowering of the PiO_2 , VO_2 stayed relatively unchanged down to values of 60–70 mm. Hg. During this decrease in the O_2 availability of the ambient water, the oxygen tension in the coelomic fluid characteristically showed two types of response patterns. In most cases $PcfO_2$ started to increase as soon as PiO_2 was lowered (Figs. 1 and 2). In other cases

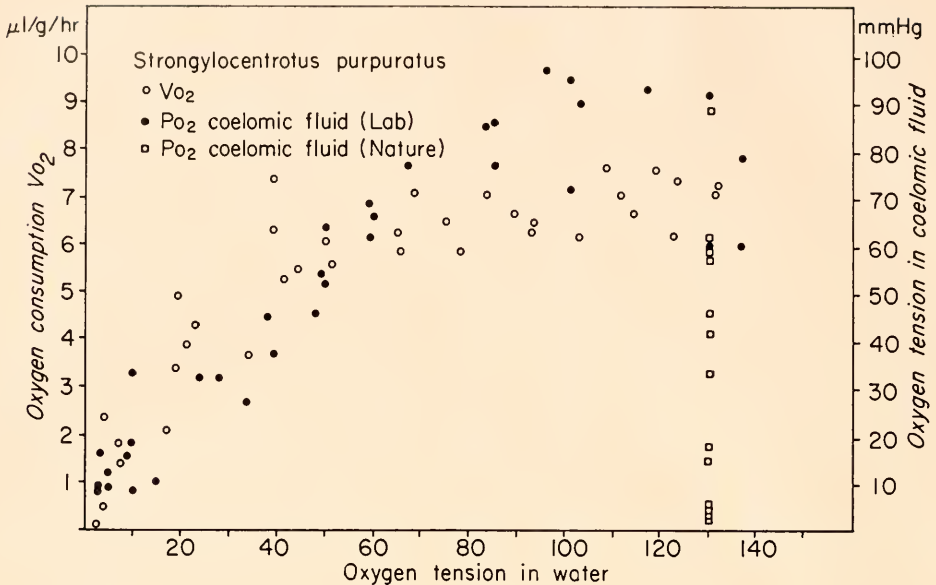


FIGURE 1. Oxygen consumption VO_2 (left ordinate, open circles), and partial pressure of oxygen in coelomic fluid, $P_{co}O_2$ (right ordinate, filled circles), of *Strongylocentrotus purpuratus* plotted against the partial pressure of oxygen in ambient water. $P_{co}O_2$ in samples obtained from animals in nature are also plotted (right ordinate, open squares).

it was maintained but in no case did $P_{co}O_2$ drop initially when P_iO_2 decreased. The increased or maintained $P_{co}O_2$ rapidly reduced the gradient in O_2 tension between the coelomic fluid and the external environment. At the breaking point in oxygen uptake, at a P_iO_2 of 60–70 mm. Hg, the internal and external media were essentially in equilibrium with respect to PO_2 . Some of the values for $P_{co}O_2$ were considerably higher than the ambient water PO_2 (Figs. 1 and 2). Inadequate stirring in the coelomic fluid space may have been responsible for the seemingly paradoxical situation. However, Newell and Courtney (1965), working on respiratory movements in a holothurian, observed a similar response and maintained that the increased oxygen concentration of the coelomic fluid resulted from the animal's ability to absorb water and from a delayed transfer of oxygen from the respiratory trees to the coelomic fluid. Such an explanation, however, seems unlikely in the case of sea urchins.

When the P_iO_2 dropped below values of 60–70 mm. Hg, both VO_2 and $P_{co}O_2$ dropped sharply and at about the same rate (Figs. 1 and 2). At very low oxygen tensions in the ambient water (19–20 mm. Hg) the VO_2 and $P_{co}O_2$ started to level off and were observed to remain largely unchanged at that low P_iO_2 for periods of at least 5–6 hours. VO_2 was then reduced to approximately 15% of its value in air-saturated water. All animals subjected to experiments of this nature completely recovered after being transferred back to aerated sea water.

Figure 3 shows the composite results (average of 4 runs on each species) from experiments designed to study the gas exchange of sea urchins when placed in air.

The oxygen tension in the coelomic fluid followed a similar course for all three species. During the first hour after transfer to air, the $P_{cf}O_2$ fell rapidly but subsequently levelled off and remained relatively unchanged from the second to at least the twelfth hour after initial exposure to air. All experimental animals recovered after being returned to normal sea water. The water and air temperatures during these experiments were 10° C. and 17° C., respectively.

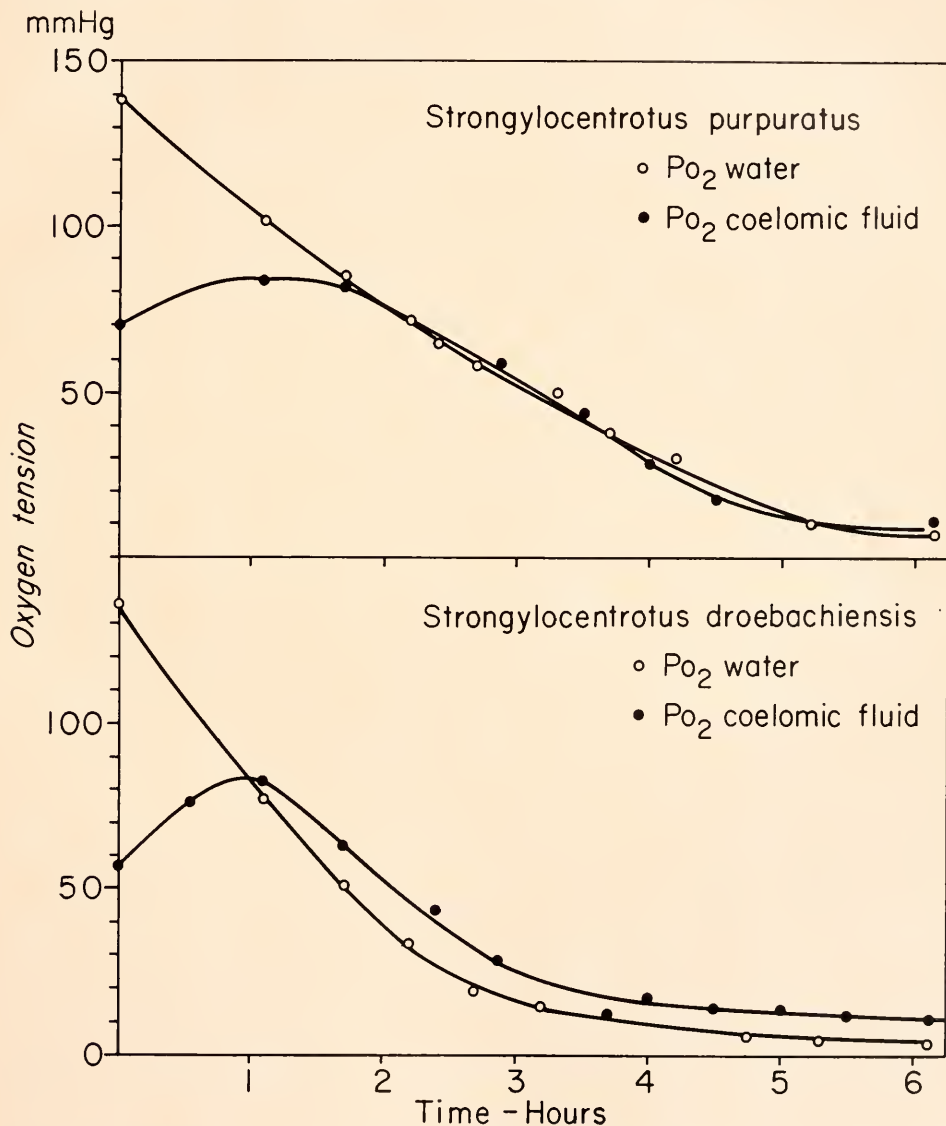


FIGURE 2. Partial pressures of oxygen in ambient water and coelomic fluid plotted against time for *S. purpuratus* and *S. droebachiensis*, when the urchins were in a closed respiration chamber.

DISCUSSION

The water vascular system of echinoids constitutes their primary means for external gas exchange (Koller and Meyer, 1933, Steen, 1965). The system terminates externally in the podia or tube feet which make up the major surface for gas exchange. The external surface of echinoids is irrigated by ciliary currents. In addition, the movement of the tube feet increases the circulation of the external water. The water vascular system is lined internally by a flattened ciliated epithelium responsible for maintaining the water current carrying the respiratory gases. However, echinoids do not possess an effective system for internal oxygen transport.

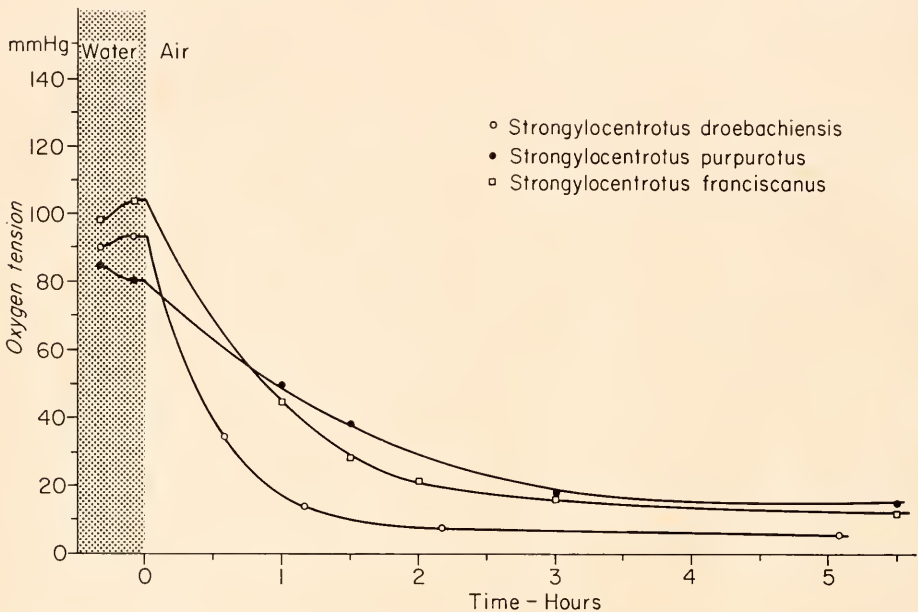


FIGURE 3. Partial pressure of coelomic fluid during air exposure of *S. droebachiensis*, *S. purpuratus* and *S. franciscanus*.

A large coelomic space lined with a ciliated epithelium constitutes the link between the water vascular system and the main mass of metabolizing tissue suspended within the spacious coelomic compartment. Although this arrangement does not insure an effective rate of transfer of oxygen from the ambient water to the respiring tissues, one must keep in mind the obvious role of the large coelomic fluid space as a storage compartment for the respiratory gases. Internal circulation based on fluid movement in discrete vessels and capillary beds does not offer a comparable storage factor. The importance of this in intertidal forms is amplified when considering the long interruptions of effective external gas exchange during tidal exposure. The role of the storage factor in respiratory exchange in holothurians has recently been alluded to by Newell and Courtney (1965).

The present data show that the oxygen tension of the coelomic fluid offers a good indication of the oxygen uptake of the animal, *i.e.*, that the rate of aerobic

metabolism closely follows the oxygen availability in the internal environment bathing the metabolizing tissues. This in essence is carrying the concept of respiratory dependence a step further using the internal rather than the external environment as the reference for the oxygen uptake. Additional evidence for the close dependence of $\dot{V}O_2$ on the PO_2 of the coelomic fluid was obtained in experiments involving exposure of the urchins to hyperoxygenated water (PiO_2 : 350–500 mm. Hg). This procedure led to a rapid increase in $PcfO_2$ to values approaching that in the ambient water. Simultaneous monitoring of the oxygen uptake showed a conspicuous increase. It was imperative to extend these experiments over a long period of time (10–15 hours) in order to separate the true level of oxygen uptake from the mere storage of oxygen in the spacious coelomic fluid compartment.

Giese *et al.* (1966) suggest a similar dependence of $\dot{V}O_2$ on the internal oxygen concentration, but their experiments were not designed to offer direct information on the problem.

The physiological significance of the close correlation between $\dot{V}O_2$ and $PcfO_2$ becomes paramount in view of our data concerning the PO_2 levels in coelomic fluid sampled from animals in their normal environment. These data (represented by the squares in Figure 1) demonstrate a very large variation in $PcfO_2$ which in turn would indicate a similar variability in the actual oxygen uptake. One must now remember that all of these samples of coelomic fluid were obtained from animals located in water essentially air-saturated with oxygen ($PiO_2 > 130$ mm. Hg). Thus, in spite of a high O_2 availability in the ambient water the animal may decrease external respiratory efforts with a consequent reduction of coelomic fluid PO_2 and a reduction of overall O_2 uptake. In other words, rather than having its metabolic rate changed at the mercy of the external environment, the animal can make use of the phenomenon of respiratory dependence by lowering its internal O_2 level in well oxygenated water and thus reduce the oxygen uptake, thereby conserving energy when it is not needed.

The applicability of this concept, in a larger biological sense, seems rather important in many lower forms where only intermittent needs for a high operational ability are present, for instance, during feeding, escape and reproductive activities or in cycles depending on internal biochemical events.

The actual values for oxygen uptake of *S. purpuratus* obtained in the present study are much lower than those reported by Giese *et al.* (1966), but they compare well with the value listed for *S. lividus* (Spector, 1956).

The tolerance to air exposure as demonstrated in the present study is relevant to the problem of intertidal distribution of sea urchins. Our results show that during exposure to moist air at moderately increased temperatures (5° C. maximum increase) the external gas exchange can support a steady oxygen uptake at a level approximately 1/5 to 1/7 of the maximum in aerated water. Periods up to 15 hours of exposure to the described conditions were compatible with survival for the species investigated. The data warrant the suggestion that the endurance limit to air exposure at low tide may be more dependent on temperature increases and desiccation than on the ability of the urchins to exchange gases with air.

The capacity for compensatory alteration of the external gas exchange by means of the water vascular system became evident from the experiments involving gradual exposure to hypoxic water (Fig. 2). All animals responded to this by a compensa-

tion that maintained or even increased the P_{cfO_2} as the P_{iO_2} dropped. It is interesting to consider this compensation on the background of recent findings by Steen (1965) who showed that the oxygen uptake measured during the course of his experiments on *Strongylocentrotus droebachiensis* was only about one-tenth of the theoretical capacity of their podia. Steen relates this apparent inefficiency to limitations in the actual transport mechanisms of external and internal media. A compensation as demonstrated seems all the more likely when the limitation in external gas exchange is set by the movement of the respiratory media rather than by the thickness and area of the exchange surface.

The assistance of Stewart Grant and Dennis Willows in the diving operations of this study and the thoughtful comments by Dr. David Hanson are greatly appreciated.

SUMMARY

1. Oxygen uptake (VO_2) by sea urchins has been measured and correlated with partial pressures of oxygen in the ambient water (P_{iO_2}) and in coelomic fluid (P_{cfO_2}). P_{cfO_2} was also analyzed in samples obtained from animals in natural environments using SCUBA-diving technique. In addition, changes in P_{cfO_2} were recorded during prolonged air exposure of the animals.

2. The three species investigated, *Strongylocentrotus purpuratus*, *S. franciscanus* and *S. droebachiensis*, showed steady levels of VO_2 until P_{iO_2} had dropped to 60–70 mm. Hg. At lower P_{iO_2} the oxygen uptake decreased corresponding to the rate of decline of P_{iO_2} .

3. Changes in P_{cfO_2} at decreasing P_{iO_2} closely paralleled the changes in VO_2 , except for a common initial compensatory increase in P_{cfO_2} . The data indicate that P_{cfO_2} represents a useful index of the level of VO_2 .

4. P_{cfO_2} in samples obtained from urchins in their natural habitat showed large variations. Means by which the relationship between P_{cfO_2} and VO_2 may actively be used by the animals in their energy economy are discussed.

5. Air exposure of the urchins while monitoring changes in P_{cfO_2} suggests that external gas exchange in air is not a critical survival factor during tidal exposure.

LITERATURE CITED

- GIESE, A. C., A. FARMANFARMAIAN, S. HILDEN AND P. DOEZEMA, 1966. Respiration during the reproductive cycle in the sea urchin, *Strongylocentrotus purpuratus*. *Biol. Bull.*, **130**: 192–201.
- HANDBOOK OF BIOLOGICAL DATA, 1956. Ed. W. S. Spector, W. B. Saunders Co., Philadelphia and London: 584 pp.
- KOLLER, G., AND H. MEYER, 1933. Versuche über die Atmung der Echinodermen (*Asterias rubens* und *Echinus miliaris*). *Biol. Zbl.*, **53**: 655–661.
- LENFANT, C., 1961. A method for measuring VO_2 and VCO_2 of very small sea animals. *J. Appl. Physiol.*, **16**: 768–770.
- NEWELL, R. C., AND W. A. M. COURTNEY, 1965. Respiratory movements in *Holothuria forskali* (Delle Chiaje). *J. Exp. Biol.*, **42**: 45–57.
- STEEN, J. B., 1965. Comparative aspects of the respiratory gas exchange of sea urchins. *Acta Physiol Scand.*, **63**: 164–170.